U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-256 334

Chronic Toxicity of Lindane to Selected Aquatic Invertebrates and Fishes

EG and G, Bionomics

Prepared For
Environmental Research Lab.

May 1976

230098

PB 256 334

EPA-600/3-76-046
May 1976

Ecological Research Series

FORCES AFFECTING THE ABILITY OF LIMONITE TO REMOVE PHOSPHATES AND FLUORIDES

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-76-046		

4. TITLE AND SUBTITLE
Chronic Toxicity of Lindane to Selected Aquatic Invertebrates and Fishes

5. REPORT DATE
May 1976 (Issuing Date)

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Kenneth J. Macek, Kenneth S. Buxton, Steven K. Derr, J. W. Dean, Scott Sauter

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Bionomics
EG & G Inc.
790 Main Street
Wareham, Massachusetts 02571

10. PROGRAM ELEMENT NO.
1BA608

11. CONTRACT/GRANT NO.
68-01-0154 and 68-01-1841

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory-Duluth
Office of Research and Development
U.S Environmental Protection Agency
Duluth, Minnesota 55804

13. TYPE OF REPORT AND PERIOD COVERED
Final

14. SPONSORING AGENCY CODE
EPA-ORD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Representatives of the aquatic invertebrate species of water flea (Daphnia magna), midge (Chironomus tentans), and scud (Gammarus fasciatus; and the fish species of bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), and brook trout (Salvelinus fontinalis) were chronically exposed to various concentrations of lindane in separate flowing water systems.

Maximum acceptable toxicant concentrations (MATC) of lindane for the selected species in soft water were estimated using survival, growth, and reproduction as indicators of toxic effects. The MATC was estimated to be between 2.2 and 5.0 µg/l for midges, between 11 and 19 µg/l for the water flea, and between 4.3 and 8.6 µg/l for the scud. For fishes the MATC was estimated between 9.1 and 12.5 µg/l for bluegills, between 9.1 and 23.5 µg/l for fathead minnows, and between 8.8 and 16.6 µg/l for brook trout. The incipient lethal concentration (LC50) for fishes and the 48-hour LC50 for invertebrates was estimated from acute exposures and used to calculate application factors (MATC/LC50). For aquatic invertebrates and lindane the estimated application factors were between 0.010 and 0.024 for midges, between 0.020 and 0.029 for water flea, and between 0.11 and 0.22 for scud. Application factors were estimated between 0.30 and 0.42 for bluegill, between 0.13 and 0.34 for fathead minnows, and between 0.34 and 0.64 for brook trout.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicity Fishes Invertebrates Pesticides	Chronic toxicity Tissue residues Flowing water system Application factors Lindane	06T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release unlimited	Unclassified	58
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	4.50

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1976-657-695/5448 Region No. 5-11

PRICES SUBJECT TO CHANGE

EPA-600/3-76-046
May 1976

CHRONIC TOXICITY OF LINDANE TO SELECTED AQUATIC INVERTEBRATES AND FISHES

by

Kenneth J. Macek
Kenneth S. Buxton
Steven K. Derr
J. W. Dean
Scott Sauter

Bionomics, EG&G Inc.
Wareham, Massachusetts 02571

Contract No. 68-01-0154
68-01-1841

Project Officer

John G. Eaton
Environmental Research Laboratory
Duluth, Minnesota 55804

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
DULUTH, MINNESOTA 55804

iv

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

Representatives of the aquatic invertebrate species of water flea (*Daphnia magna*), midge (*Chironomus tentans*), and scud (*Gammarus fasciatus*); and the fish species of bluegill (*Lepomis macrochirus*), fathead minnow (*Pimephales promelas*), and brook trout (*Salvelinus fontinalis*) were chronically exposed to various concentrations of lindane in separate flowing water systems.

Maximum acceptable toxicant concentrations (MATC) of lindane for the selected species in soft water were estimated using survival, growth, and reproduction as indicators of toxic effects. The MATC was estimated to be between 2.2 and 5.0 µg/l for midges, between 11 and 19 µg/l for the water flea, and between 4.3 and 8.6 µg/l for scud. For fishes the MATC was estimated between 9.1 and 12.5 for bluegills, between 9.1 and 23.5 µg/l for fathead minnows, and between 8.8 and 16.6 µg/l for brook trout. The incipient median lethal concentration (LC50) for fishes and the 48-hour LC50 for invertebrates was estimated from acute exposures and used to calculate application factors (MATC/LC50). For aquatic invertebrates and lindane the estimated application factors were between 0.010 and 0.024 for midges, between 0.020 and 0.029 for water flea, and between 0.11 and 0.22 for scud. Application factors were estimated between 0.30 and 0.42 for bluegill, between 0.13 and 0.34 for fathead minnows, and between 0.34 and 0.64 for brook trout.

This report was submitted in fulfillment of Project Number 18050 HQH, Contracts 68-01-0154 and 68-01-0841 by Bionomics, E G & G, Inc. under the sponsorship of the Water Quality Office, Environmental Protection Agency. Work was completed as of February 1974.

CONTENTS

SECTION		PAGE
I	Conclusions	1
II	Recommendations	2
III	Introduction	4
IV	Materials and Methods	6
	Chronic Exposure Systems	6
	Acute Toxicity Procedures	10
	Chemical Methods	10
	Statistics	12
	Chronic Exposure	13
	Chironomus tentans	13
	Daphnia magna	13
	Gammarus fasciatus	14
	Lepomis macrochirus	15
	Pimephales promelas	16
	Salvelinus fontinalis	17
V	Results	20
	Acute Bioassays	20
	Water Chemistry	20
	Chronic Exposure	23
	Chironomus tentans	23
	Daphnia magna	24
	Gammarus fasciatus	26
	Lepomis macrochirus	28
	Pimephales promelas	30
	Salvelinus fontinalis	33
	Residue Analysis	37
	Calculation of Application Factors	37
VI	Discussion	40
VII	References	46

TABLES

		PAGE
1.	Physical characteristics of test chambers utilized to evaluate the chronic toxicity of lindane to fishes and fish food organism	7
2.	Chemical analysis of the diluent water utilized during chronic aqueous exposure of fishes and aquatic invertebrates to lindane	9
3.	Percent recovery of added lindane from water (µg/l) and whole fish (µg/g)	12
4.	Mean and range of measured concentrations of hardness, alkalinity, acidity, dissolved oxygen and pH from water samples taken periodically during chronic exposure of aquatic invertebrates and fishes to lindane	21
5.	Nominal and measured lindane concentrations (µg/l) in water during chronic exposure of aquatic invertebrates and fishes to lindane	22
6.	Summary of the effect of various concentrations of lindane on hatchability, pupation and emergence of *Chironomus tentans* continuously exposed for two generations	23
7.	Mean percent survival of *Daphnia magna* continuously exposed to lindane for 64 days	25
8.	Mean production of young per female *Daphnia magna* continuously exposed to lindane for 64 days	26
9.	Survival and reproductive success of *Gammarus fasciatus* exposed to lindane for 17 weeks	27
10.	Survival and growth during 6 and 18 months, and results of spawning activity of bluegill (*Lepomis macrochirus*) continuously exposed to lindane	29
11.	Hatchability, survival and growth of bluegill (*Lepomis macrochirus*) fry exposed to lindane	30
12.	Survival and growth of fathead minnows (*Pimephales promelas*) during 30 days, 60 days, and 43 weeks continuous exposure to lindane	31

PAGE

13. Sexual development, spawning, hatchability of eggs, and survival and growth of offspring after 30 and 60 days, for fathead minnow (Pimephales promelas) continuously exposed to lindane 32

14. Growth of yearling brook trout (Salvelinus fontinalis) during 261 days continuous exposure to lindane 34

15. Results of spawning activity of yearling brook trout (Salvelinus fontinalis) during continuous exposure to lindane 35

16. Survival and growth of second generation brook trout (Salvelinus fontinalis) during the first 90 days of development of fry continuously exposed to lindane 36

17. Mean measured lindane concentration in water (µg/l) and in the muscle (µg/kg) of bluegill and brook trout and in the eviscerated carcass (µg/kg) of fathead minnows continuously exposed to lindane 38

18. Summary of concentrations of lindane (µg/l) producing acute and chronic toxicity to aquatic species, and calculated application factors describing the relationship between acute and chronic toxicity 39

19. Summary of application factors reported for chemicals to various species of fish 45

ACKNOWLEDGEMENTS

During the period of time devoted to this research effort, we were fortunate to have laboratory assistance from Mark Lindberg and Gerald LeBlanc, who maintained exposure systems and were significantly involved with routine aspects of the fish chronics. Gratitude is extended to Sarah Gnilka, Pat Costa and Karen Frey for their involvement and expertise in conducting the invertebrate chronics. Also, our appreciation is directed to Curtis Hutchinson for construction of all diluter systems utilized throughout this study, and to Patrick (Rod) Parrish for constructively reviewing the final report.

We are grateful to Mr. Richard Griffith, Northeast Regional Director, U.S. Bureau of Sport Fisheries, Boston, Mass, for his assistance in obtaining disease-free brook trout used in the chronic exposure and to Mr. Quentin Pickering, Newtown Fish Toxicology
Cincinnati, Ohio for providing fathead minnow eggs used in the study.

Finally, our sincere appreciation is extended to John Eaton, Project Officer, Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minnesota, for his guidance and constructive advice during the performance of these studies.

SECTION I

CONCLUSIONS

Estimates (LC50) of the acute toxicity of lindane to the species tested ranged from 26-76 µg/l for bluegills, fathead minnows, brook trout and gammarids, while the range of estimated acute toxicity of lindane to daphnids and midges was generally an order of magnitude higher (207-485 µg/l).

Of the six species studied during chronic bioassays, the midges and gammarids were the most sensitive to lindane. Estimated limits of the maximum acceptable toxicant concentrations (MATC) of lindane for these two invertebrate species were within the range of 2.2-8.6 µg/l.

All of the fish species studied were similar in their susceptibility to chronic exposure to lindane. Estimated limits of the MATC of lindane for the fishes were within the range of 8.8-23.5 µg/l, as were those for daphnids.

The toxicity of lindane to the invertebrate species was generally cumulative from generation to generation with many instances of significant effects being observed at concentrations which apparently did not affect the previous generation.

These data suggest that populations of essential fish food organisms may be significantly reduced in numbers by exposure to concentrations of lindane which would apparently not directly affect fish populations.

Calculated limits of application factors for the three fishes were similar and were within the range of 0.13-0.64. Comparable values for the invertebrate species were within the range of 0.01-0.22. These data suggest that the concept of the use of application factors for estimating the MATC of chemicals to aquatic organisms based on the results of acute bioassays may be valid within broad taxonomic groups.

SECTION II

RECOMMENDATIONS

Of the species studied, midges (Chironomus tentans) were among the most susceptible to the toxicant. This observation, combined with the adaptability of these organisms to laboratory systems, suggests this species as a desirable bioassay organism for evaluating the chronic toxicity of chemicals to aquatic organisms.

Conversely, although scud (Gammarus fasciatus) appeared to be as sensitive as midges, it is possible this observation is related to the inability of these organisms to adapt to laboratory culture and handling. We believe much work needs to be done to develop methods of handling and testing these organisms in the laboratory which will provide better survival and reproduction under these conditions. Such methods would allow for more sensitive comparison between the response of treated and control groups to toxicant exposure.

To a lesser degree, we feel the same recommendation should be made regarding bluegill. More information regarding the laboratory conditions necessary to induce and maintain survival and spawning activity, and the procedures necessary to successfully handle and feed newly hatched bluegill fry, must be developed to enable successful completion of bluegill chronic toxicity bioassays in a reasonable period of time.

We recommend that the fathead minnow be considered the fish species of choice, among those tested, for chronic toxicity bioassays. This recommendation is based on the fact that an adequate level of spawning activity can be induced under laboratory conditions, successful handling of eggs and larvae is possible, and excellent survival of fry is obtained. The combination of these factors provides opportunities for statistically and biologically sensitive evaluation of possible toxic effects due to chronic exposure to chemicals. Finally, studies with this species represent the only "true chronic" (at least one complete life history) of the fishes studied.

Because of the cumulative nature of the toxicity of lindane to several invertebrate species, it appears that some information based on multi-generation studies is necessary to adequately assess the potential long-term effects of the pesticide to these species. We suggest that similar multi-generation studies may be necessary with at least one fish species to assess the possible cumulative effects of lindane on fish.

In view of the order of magnitude difference between application factors calculated for fishes and those calculated for two of the aquatic invertebrates, we suggest that future laboratory investigations to assess the chronic toxicity of chemicals to aquatic organisms consider at least one fish species and one of the more sensitive invertebrate species.

Tissue residue information on fishes, or fish fry, based on a single interval during the life history provides limited information on the subject of bioaccumulation. We suggest that by including additional fish at the initiation of a chronic exposure, and sampling periodically during the first 60-90 days of exposure, additional significant information regarding the uptake, accumulation and retention of chemical residues by fish would be generated. It is possible that this information could then be correlated with observed biological responses to chemical exposure.

SECTION III

INTRODUCTION

The current concern regarding the protection of aquatic life in surface waters has prompted evaluation of the effects of chemicals on aquatic invertebrates and fishes. Much of the toxicological research on aquatic biota has been limited to the development of acute toxicity values as a measure of the biological effect of these compounds. More recently, utilization of the chronic exposure of fishes to chemicals has received particular attention due to the numerous parameters that can be evaluated as indices of toxic effects of a particular compound (Mount, 1968; Mount and Stephan, 1967; Eaton, 1970; McKim and Benoit, 1971; Hermanutz et al., 1973). The "Laboratory Fish Production Index (LFPI)" as defined by Mount and Stephan (1967) reflects toxic effects on reproduction, growth, spawning behavior, egg mortality, and fry survival. The highest observed toxicant concentration that has no effect on these parameters during continuous chronic exposure is termed the maximum acceptable toxicant concentration (MATC).

Lindane, the gamma isomer of benzene hexachloride (1,2,3,4, 5,6-hexachlorocyclohexane), is one of the few chlorinated hydrocarbon insecticides still widely used for agricultural purposes, particularly as a cereal seed dressing (Brooks, 1972). Current pesticide monitoring programs indicate that lindane is present in the Mississippi River and some streams in the Western U.S., at concentrations ranging from 2.8-28.0 ng/l (Matsumura, 1972). Past studies have demonstrated that lindane is relatively persistent in natural waters and sediment and that significant degradation occurs only under anaerobic conditions (Brooks, 1972; Nash and Woolson, 1967). Although the persistence of lindane could pose a significant environmental hazard, studies to date have indicated that the biological accumulation and persistence of lindane is low when compared to compounds such as DDT and dieldrin (Wilson, 1965; Gakstatter and Weiss, 1967).

Past studies with lindane have consisted mainly of low-dose feeding experiments or short-term aqueous exposures which generated acute toxicity values. This study was undertaken to determine the MATC for selected aquatic invertebrates and fishes based on continuous chronic exposure. The organisms selected for this research effort were:

INVERTEBRATES

1. Chironomus tentans (Chironomidae, mdige)
2. Daphnia magna (Cladocera, water flea)
3. Gammarus fasciatus (Gammaridae, scud)

FISHES

1. Lepomis macrochirus (Centrarchidae, bluegill)
2. Pimephales promelas (Cyprinidae, fathead minnow)
3. Salvelinus fontinalis (Salmonidae, brook trout)

A reason for selecting both invertebrates and fishes is that the susceptibility of fishes to a chemical should be compared to the susceptibility of fish-food organisms to that same chemical. An understanding of both these phenomena will be invaluable in establishing realistic and meaningful water quality criteria and standards.

SECTION IV

MATERIALS AND METHODS

The methodology for chronic testing of fishes generally followed the recommended bioassay procedures issued by the Environmental Protection Agency's Environmental Research Laboratory-Duluth, Duluth, Minnesota (Bioassay Committee, 1971a) 1971b, 1971c). Acute bioassay procedures were generally those recommended in Standard Methods for the Examination of Water and Wastewater (1971). Chronic testing procedures for invertebrates were determined through communication of Bionomics staff members with personnel at the Environmental Research Laboratory-Duluth.

CHRONIC EXPOSURE SYSTEMS

Proportional diluters (Mount and Brungs, 1967), with a dilution factor of 0.5 and a syringe injector, delivered the test water and toxicant, dissolved in dimethyl sulfoxide, to the mixing chamber, mixing cells, and ultimately to the test chambers. Five lindane concentrations and a control flowed to mixing containers and into separate glass delivery tubes leading to the replicate test chambers. In the test system for Daphnia magna, baffles were inserted in each of the quadruplicate test chambers to minimize turbulence of influent water. The diluter was also modified to include food cells which delivered a measured amount of food along with the toxicant and diluent water. All other exposure systems utilized duplicate test chambers of varying construction and flow rates as summarized in Table 1.

Table 1. PHYSICAL CHARACTERISTICS OF TEST CHAMBERS UTILIZED TO EVALUATE THE CHRONIC TOXICITY OF LINDANE TO FISHES AND FISH FOOD ORGANISMS

Species	Material (shape)	Dimensions[a] (cm)	Water Depth (cm)	Volume (liters)	Flow Rate (tank vol./ 24 hours)
Chironomus tentans	glass (rectangular)	21x26x18	16.0	7.5	6
Daphnia magna	glass (cylindrical)	17x13.5	14.0	1.8	2
Gammarus fasciatus	glass (rectangular)	25x40x21	19.0	16.0	3
Lepomis macrochirus	stainless steel (rectangular)	40x180x30.5	30.5	168.0	7
Pimephales promelas	glass (rectangular)	30.5x90x30.5	15.0	41.0	7
Salvelinus fontinalis	stainless steel (rectangular)	40x90x30.5	30.5	84.0	7

[a]Dimensions are height x length x width for rectangular and height x diameter for cylindrical chambers.

Two growth chambers 21 x 20 x 15 cm (height x length x width) with a water depth of 16 cm were provided for the young Gammarus and received test water at a flow rate equal to that in the adult test chambers. Fathead minnow test chambers were subdivided to provide space for two growth chambers 25 x 20.5 x 12.5 cm, and test chambers for brook trout contained a shelf which supported two growth chambers 25 x 25 x 12.5 cm with a water depth of 12.5 cm. Test water was delivered directly to the test chamber and growth chambers through a glass, flow-splitting chamber calibrated to keep the flow rate equal in all chambers. The bluegill test chambers supported two 18 x 28 x 12 cm growth chambers with a water depth of 10.5 cm; and an air-driven system was utilized to pump water from the test chamber to the growth chambers at a rate of 6 chamber volumes every 24 hours. For all fish tests, 40 mesh stainless steel screen was affixed to one end of each growth chamber to allow water to flow out while retaining the young fish. Bluegill and brook trout test chambers were aerated with oil-free air in order to maintain dissolved oxygen levels above 60 percent saturation.

Diluent water was pumped from a 400 foot subterranean well to a cement holding tank. Results of the chemical analysis of the diluent water are summarized in Table 2. Diluent water was delivered through PVC pipes to the exposure systems. Unheated well water was mixed with heated well water from a glass-lined heater to provide a constant temperature of 28°C before delivery to the bluegill experimental unit. Test chambers in the fathead minnow and brook trout units were maintained in circulating water baths at the desired test temperatures. Water circulating in the water baths of the fathead minnow unit was heated by two immersion coil heaters controlled by a mercury column thermoregulator which maintained a temperature of 25 ± 1°C. Water in the baths of the brook trout unit was cooled by recirculating through a mechanical chiller to temperatures of 15 ± 1°C from May through August, 12 ± 1°C during September, and 9 ± 1°C from October through the end of juvenile exposure. Prior to entering experimental units for the invertebrate species, diluent water was delivered to a stainless steel headbox where immersion coil heaters and thermoregulators maintained a temperature of 19 ± 1°C for Daphnia and Gammarus and 23 ± 1°C for Chironomus. In addition, ultra-violet lights (24 watt) were placed over the headbox and water cells of each invertebrate diluter to minimize the introduction of

Table 2. CHEMICAL ANALYSIS OF THE DILUENT WATER UTILIZED DURING CHRONIC AQUEOUS EXPOSURE OF FISHES AND AQUATIC INVERTEBRATES TO LINDANE

Parameter	mg/liter	Parameter	mg/liter
Calcium	6.0	Chloride	17.6
Magnesium	2.1	Fluoride	0.5
Potassium	1.1	Cyanide	<0.005
Sulfate	11.6	Iron	<0.01
Nitrate	<0.05	Copper	0.004
Nitrite	<0.05	Zinc	0.01
Ammonia	0.01	Cadimum	0.001
Phenol	<0.001	Chromium	<0.001
Chlorine	<0.01	Lead	<0.01

fungus and pathogens into the test system.

Illumination was provided by a combination of Durotest (Optima FS) and wide spectrum Grow Lux fluorescent lights fixed centrally above the test chambers in all experimental units. Incandescent bulbs (100 watt) simulated a 15-minute dawn or dusk light intensity change (Drummond and Dawson, 1970). A constant 16-hour light, 8-hour dark photoperiod was controlled by an automatic timer in the Chironomus and Daphnia experiments. The photoperiod for Gammarus, bluegills, and brook trout followed the normal daylight hours of Evansville, Indiana (representative U.S. daylength), and was adjusted the first and fifteenth of each month. The photoperiod for fathead minnows followed Evansville daylengths but was started with the daylength for December 1st on day one of the experiment (Nov. 11). All experimental units were screened with black polyethylene curtains to prevent unnecessary disturbance of test organisms and the influence of extraneous lighting on the intended photoperiod.

ACUTE TOXICITY PROCEDURES

Static acute toxicity bioassays were conducted with invertebrates and lindane to estimate the 48-hour LC50 and its 95% confidence interval. Five organisms in three of four replicate containers at each concentration were exposed at 20 ± 1°C. First instar Chironomus tentans, third instar Gammarus fasciatus, and <24 hour old Daphnia magna were the organisms exposed. A linear regression equation was calculated after converting test concentrations and corresponding percent mortalities to logarithms and probits, respectively, and this equation was utilized to estimate the 48-hour LC50's and confidence intervals.

Acute toxicity studies with fishes and lindane were conducted in flow-through water systems using proportional diluters (Mount and Brungs, 1967). The incipient LC50 was estimated when no additional significant mortality (>10%) of the test organisms was observed at any concentration for 48 hours. At this time, the exposure was terminated and a linear regression equation was calculated by converting lindane concentrations and corresponding mortalities into logarithms and probits, respectively. This equation was utilized to estimate the incipient LC50 and 95% confidence interval.

The chronic exposure concentrations for both invertebrates and fishes were selected by evaluating observed mortality and no-effect concentrations from the acute studies. The 48-hour LC50 for invertebrates and the incipient LC50 for fishes were used to estimate application factors describing the relationship between acute and chronic toxicity.

CHEMICAL METHODS

Toxicant concentrations and basic water quality characteristics were initially monitored in each aquarium each week to establish that the concentrations and water quality characteristics were constant with minimum variability. After the determination of what these concentrations and characteristics actually were in the experimental systems, a minimum monitoring effort was conducted to measure variability and detect changes from the established means. Stock solutions of lindane (100% a.i., City Chemical Co., N.Y.) in nanograde dimethyl sulfoxide (DMSO) were delivered to the dilution water from 50 ml glass syringes with stainless steel needles. The solvent (DMSO) was not added to the control water in any of the chronic exposures and the amount added to the highest lindane concentration of each chronic test ranged from 6 µg/l during the brook trout

chronic to 65 µg/l during the Daphnia chronic. In each case, these concentrations were determined to be less than 1/500 of the 96-hour LC50 of the solvent for each species. Generally, lindane concentrations were determined once each week during each of the chronic exposures by taking 500-ml water samples from each aquarium.

All glassware used in the analysis of samples was cleaned according to methods described in the Analytical Handbook for Water Quality Control in Water and Wastewater Laboratories (U.S. EPA, 1972). Water samples (500 ml) were extracted with three 50 ml portions of methylene chloride, dried with anhydrous sodium sulfate (heated and purified), and evaporated in a Kuderna-Danish evaporator, according to the method described by U.S. EPA (1971). The resulting extract was dissolved in hexane and an aliquot analyzed by gas chromatography using a Beckman Model 45 gas chromatograph. The glass column used was 180 cm x 2 mm containing 10 percent Dexsil 300 GC on 80/100 Supelcoport at 210° with 30 cc helium per minute at 28 psi head pressure as the carrier. A helium-arc electron capture detector operated at 255°C was used and received 110 cc helium per minute. Using an electrometer attenuation of 8×10^{-10} amps, a one-millivolt recorder produced a one half scale deflection with 0.1 ng of lindane.

Before analysis of each set of water samples, aliquots of hexane containing known amounts of lindane were chromatographed and a graph of peak heights versus nanograms of lindane was constructed as a standard curve. Nanograms of lindane present in the sample aliquots were determined by graphical interpolation. A mean recovery of 93 percent obtained from analysis of known standards (Table 3) was applied to the observed instrument response to evaluate measured lindane concentrations in the water samples.

Lindane residues were extracted from the tissues of exposed fishes by the method described by Hesselberg and Johnson (1972). Exceptions to this method were the use of 1:1 petroleum and ethyl ether as the extraction solvent, and the cleanup of the total extract rather than handling the fats and lipids separately. After weighing, the fish tissue samples were extracted in a Waring Blendor^{R} containing 1:1 petroleum and ethyl ether. The extract was filtered, evaporated, and freed of extraneous fish oils and lipids by methods described by EPA (1971). The clean extract was brought to known volume with hexane and analyzed by

gas chromatrography using the methods and materials described earlier for lindane analysis of water samples. A mean recovery of 93 percent was determined from analysis of tissue with a known amount of lindane added (Table 3). This percent recovery was applied to the observed instrument response to lindane in fish tissue to calculate measured lindane residues.

TABLE 3. PERCENT RECOVERY OF ADDED LINDANE FROM WATER (µg/l) AND WHOLE FISH (µg/g)

Sample Type	Added Conc. (ppm)	Measured Conc. (ppm)	Recovery (%)
Water	0.025	0.023	92
	0.025	0.024	96
	0.025	0.023	92
	0.125	0.123	98
	0.125	0.110	88
	0.125	0.117	94
			93 ± 3.5
Whole Fish	0.101	0.117	117
	0.164	0.150	91
	0.118	0.108	92
	1.27	1.13	89
	1.12	1.03	92
	1.77	1.37	77
			93 ± 13

During the chronic exposures, total hardness, alkalinity, pH and acidity were generally measured bi-weekly in the control and one lindane concentration according to Standard Methods for the Analysis of Water and Wastewater (APHA, 1971). Temperature and dissolved oxygen concentrations were measured in selected tanks each day using YSI dissolved oxygen meter with a combined oxygen-temperature probe.

STATISTICS

Means of the biological parameters measured in each duplicate container during chronic exposures were subjected to analysis of variance according to Steel and Torrie (1960).

When treatment effects were indicated by Anova, the means of these effects were subjected to Duncan's Multiple Range Test to determine which treatments were different from the controls. All differences were considered statistically significant at a probability of P=.05.

CHRONIC EXPOSURE

Chironomus tentans

Stock cultures of Chironomus tentans were obtained from Michigan State University, East Lansing, Michigan. Cultures were Maintained in 60 l glass aquaria utilizing a substrate prepared by placing 50 grams of paper hand towel (Nibroc, Brown Co.) in a blender with 5 grams of high protein chicken feed and 1 liter of water to cover the mixture. The mixture was homogenized for three to five minutes. This substrate was used to cover the bottom of each aquarium to a depth of 1.5 cm. Emergent adults were allowed to mate within the screened aquaria and provided a ready supply of egg masses.

Egg masses were selected from the stock cultures according to uniformity of age, and one egg cup of 100 eggs was suspended in each of the duplicate experimental aquaria. Egg cups were made from 20 ml glass vials with the bottoms cut off and replaced with 40 mesh nylon screen. After hatching was completed in all treatments (3-5 days), the number of surviving first instar larvae were counted and carefully placed onto the substrate.

After 26 to 29 days, the number of emergent adults, pupal exuvia, and dead pupa were observed and recorded. Mating of adults was observed, and egg masses produced by mating pairs in each replicate were collected and utilized to conduct second generation exposures according to the above methods. At the initiation of the second generation exposure, new substrate was placed in the experimental aquaria which would receive first instar larvae. The number of emergent adults, pupal exuvia, and dead pupa was observed from each replicate concentration and the experiment terminated.

Daphnia magna

Laboratory stocks of Daphnia magna were obtained from the University of New Hampshire, Durham, New Hampshire, and successfully cultured in the laboratory according to the methods of Biesinger and Christensen (1972).

Typically, 10 daphnids (<24 hour old) were placed into each of four replicate experimental units, resulting in a total of 40 animals per concentration. A food supply consisting of trout starter and dry-powdered Cerophyl (2:1) was prepared in an aqueous suspension (12.5 mg/ml) and delivered from a Mariotte bottle via a volumetric delivery system to a mixing chamber during each diluter cycle. The diluted food suspension was subsequently transferred to the food cells from which 25 ml (0.1 mg/ml) were delivered to each test container during each diluter cycle.

Survival and reproduction of daphnids was recorded after one, two, and three weeks. Reproduction was measured by recording the number of young in each experimental chamber weekly and discarding the progeny after weeks one and two. At the end of the third week, the number of original animals remaining was recorded, the specimens discarded, and 10 daphnids (<24 hours old) were randomly selected from each chamber to begin the second generation exposure. The same procedures were followed for the second and third generation, after which the experiment was terminated.

Gammarus fasciatus

The test organisms were collected in October 1972 from the raceway outlets at the National Fish Hatchery, North Attleboro, Massachusetts. Sexually mature adults were acclimated at 17 ± 1°C in the laboratory for a period of three weeks. After the acclimation period, females were isolated and their progeny collected. When sufficient 1 to 22 day old gammarids were available, the chronic exposure to lindane was initiated by placing 30 specimens into each replicate aquarium. During the exposure, the gammardis were fed pre-soaked maple leaves, water cress, and Elodea. Brine shrimp (Artemia) nauplii and Daphnia were also fed to the gammarids weekly.

Survival was recorded once each month by siphoning the contents of each test chamber into a pan and counting the gammarids. At the onset of reproduction, all adult chambers were checked daily for gravid females which were isolated singly in 400 ml beakers containing respective test solutions. Some females were maintained for up to 28 days in beakers with the test solution being replaced every 48 hours with fresh test solution from the appropriate experimental aquarium. Twenty to fifty young collected from isolated females in the same adult chamber on the same day were placed in the respective larval growth chamber and treatment

continued for 30 days. At the end of 30 days, survival and growth were recorded by the procedure of Clemens (1950). After a period of 17 weeks, the exposure of the original gammarids to lindane was terminated and the sex of individual specimens was determined.

Lepomis macrochirus

In December 1971, chronic exposure of bluegills to lindane was initiated using 7-10 cm fish obtained from a commercial hatchery in Wisconsin. Bluegills were acclimated to the test water for three months, after which twenty fish were randomly distributed to each test chamber. Bluegills were fed ad libitum the largest commercially prepared trout pellet which they would take in two daily feedings. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus and were brushed when algal growth became excessive. Total length and weight of each individual fish was measured at the initiation of exposure, after 90 days, and at thinning (185 days) using 100 mg/l of tricaine methanesulfonate (MS-222) to lightly anesthetize the fish.

During the first year when secondary sex characteristics were well developed, fish in each tank were separated into groups of males, females, and undeveloped fish using the shape of the urogenital opening as the main criterion for sexual differentiation (McComish, 1968). Sexually mature fish were randomly reduced to 3 males and 7 females per duplicate tank and all other fish were discarded after examination of gonadal development and measurement of total length and weight. At this time, two spawning substrates similar to those described by Eaton (1970), were placed into each duplicate tank. Substrates measured 30.5 x 40 x 5 cm with a bowl-shaped depression 25 cm in diameter and 4 cm deep. Although bluegills developed secondary sex characteristica and exhibited territorial behavior characteristic of spawning bluegill populations under natural conditions, no spawning occurred after the first year of exposure to lindane. Consequently, substrates were removed and the exposure of bluegills to lindane was continued a second year, repeating the photoperiod schedule.

During the second year of bluegill exposure, when males demonstrated territorial behavior, the substrates were replaced in the proper tanks and total length and weight was determined for each fish. Substrates were checked for eggs after 1:00 P.M. each day and eggs were brushed loose

from the substrate under a stream of test water. Eggs were also siphoned from the tank bottom after each spawn. Two hundred eggs were impartially selected from each spawn and placed in an egg cup which oscillated in the respective test water by means of a rocker arm apparatus (Mount, 1968). Eggs from each spawn were counted volumetrically by first counting a subsample of exactly 1 ml and multiplying that number by the total measured volume (ml) of spawned eggs. Eggs were allowed to settle for two minutes in the graduated cylinder before measuring. Hatchability was determined as the percent of live fry hatching out of 200 eggs.

Thirty bluegill fry from the earliest two spawnings in each tank with at least 25 percent live hatch were placed in the growth chambers. Fry from all other spawns were discarded after hatchability was determined. Fry in the growth chambers were fed ad libitum three times daily with live young zooplankton from mixed laboratory cultures of copepods, rotifers, protozoans, and brine shrimp. Cumulative mortality was calculated after 30, 60 and 90 days and total lengths of fry were determined at the same time using the photographic method of McKim and Benoit (1971). Parental bluegills were sacrificed after spawning had ceased in all tanks for three weeks. Total length, total weight, sex, and gonadal condition was determined for each fish and samples of muscle were retained for residue analysis.

Pimephales promelas

Chronic exposure of fathead minnows to lindane began in November 1971 with 15-day old fish obtained as eggs from the Newtown Fish Toxicology Station, Newtown, Ohio. Forty fish were randomly distributed to each test chamber. Cumulative mortality and total length of live fish were determined after 30 and 60 days using the photographic method of McKim and Benoit (1971). At 60 days, the number of fish in each test chamber was impartially reduced to fifteen. Fathead minnows were fed ad libitum twice daily with a commercially prepared trout starter food which was supplemented with daphnids and brine shrimp nauplii. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus and were brushed when algal growth became excessive.

The discovery of bacteria and external parasites on a few fish prompted the use of flush treatments of tetracycline hydrochloride (4 mg/l active ingredient) and a combination of malachite green and formalin (25 µl/liter of formalin

containing 3.7 g/liter malachite green crystals). These treatments were concentrated between days 95 and 116 of the experiment and were applied to all experimental units (including controls).

Due to unequal numbers of males in each tank, some of the male fish were removed after secondary sexual characteristics were well developed. Five spawning sites of halved, 3 inch transite drain tiles had been placed in each tank when fish were released from growth chambers at 60 days. The tiles were placed concave surface down at locations that minimized the chance of enounters by separate egg-guarding males. When spawning began, eggs were removed and counted after 1:00 P.M. each day. Fifty eggs from each spawn were oscillated in their corresponding test water by means of an egg cup and a rocker arm apparatus (Mount, 1968). Dead eggs were removed and counted each day until hatching was completed (3-5 days at 25°C). Percent hatch was based on the number of live fry from 50 eggs.

Forty fry from the earliest two spawns in each tank with at least 80% live hatch were placed in the respective growth chambers. Cumulative mortality and total length of live fish were determined at 30 and 60 days by McKim and Benoit's (1971) photographic method. Fry from all other spawns were discarded unless a growth chamber was later made available by termination of 60 day old fry. Finely ground starter food and brine shrimp nauplii were fed three times daily to fry in the growth chambers.

Parental fish were sacrificed after all spawning had ceased for one week. Total length, weight, sex and gonadal condition was determined for each fish and three samples per concentration of eviscerated fish were retained for residue analysis.

Salvelinus fontinalis

Chronic exposure of brook trout to lindane began in May 1972 with yearling fish obtained from the National Fish Hatchery, Manchester, New Hampshire. Distribution of fish to the test chambers was delayed by the discovery of external parasites on a few fish. The parasites were controlled by flush treatments of malachite green and formalin (25 µl/l of formalin containing 3.7 g/l of malachite green). These treatments were repeated shortly after the distribution of fish to each duplicate tank to ensure control of the parasites. Brook trout were fed twice daily with a measured ration of the largest trout pellet they would take. The

feeding rate was based on a percentage of the initial average biomass per tank and was adjusted after 90 days and 186 days when total weights of fish were again measured. Feeding rates were from a New York State fish hatchery feeding chart (Deuel et al., 1952). Total length and weight of individual fish were measured at the initiation of exposure, after 90 days, and at thinning (186 days) utilizing 100 mg/l of tricaine methanesulfonate (MS-222) to quiet the fish during measuring. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus and were brushed when algal growth became excessive.

Secondary sexual characteristics were observed on test day 186 and males, females and undeveloped fish were identified in each tank and the number of sexually mature fish was randomly reduced to 2 males and 4 females per tank. All other fish were discarded after being measured and examined for gonadal condition. At this time, two stainless steel spawning substrates similar to those described by Benoit (1974) were placed in each duplicate tank. The box-like substrates 25 x 25 x 15 cm had on their bottom a gridwork of 2.5 cm cubicles, made of 20 mesh stainless steel screen, to retain the eggs where they were deposited. A 25 x 25 cm square of 4 mesh stainless steel screen was placed on top of the egg retainer and silicone adhesive was used to attach 1.3 - 2.5 cm diameter stream gravel 2 cm apart on the screening.

Substrates were checked after 1:00 P.M. each day and eggs were recovered by removing the gravel screen and egg retainer, and pipetting the eggs into a pan containing the test water. Eggs from each spawn were counted and two egg cups containing a group of 50 and 100 eggs were attached to the rocker apparatus (Mount, 1968) for incubation in their respective test water. Dead eggs were removed and recorded daily and the number of fry hatching each day in the incubation cups with 50 eggs was recorded. After 15 days, the group of 100 eggs was removed and the percentage of eggs developing a neural keel was recorded as an indication of percent fertilization. Hatchability (percent of 50 eggs) and mean time to hatch were determined after completion of hatching.

Twenty five fry from the earliest two spawns in each tank with at least 50% live hatch were placed in the respective growth chambers. Fry from all other spawns were measured and discarded after hatchability determinations.

Cumulative mortality and total length of live fry was determined at the initiation of exposure and after 30, 60 and 90 days using the photographic method of McKim and Benoit (1971). Brook trout fry were fed five times daily ad libitum with a commercially prepared trout starter food.

After spawning had ceased in all tanks for a period of three weeks, parental fish were removed and examined for gonadal condition. Total lengths and weights were determined for each individual fish before retaining muscle samples for residue analysis and discarding the remaining fish.

SECTION V

RESULTS

ACUTE BIOASSAYS

The analysis of the results of acute static bioassays with the invertebrate species indicated the 48-hour LC50 (95% confidence interval) of lindane for Chironomus, Daphnia, and Gammarus was 207 (157-263), 485 (301-623), and 39 (27-56) µg/l, respectively. Lindane-induced mortality was observed with chironomids at 180 µg/l, with daphnids at 310 µg/l, and with gammarids at 36 µg/l. Based on these data, the highest nominal concentrations selected for the chronic exposures were 24 µg/l (Chironomus), 200 µg/l (Daphnia) and 20 µg/l (Gammarus).

Duplicate continuous-flow bioassays were conducted at 25 ± 1°C with 0.75 gram bluegill. The 96-hour LC50 values for lindane in both instances were >100 µg/l. The earliest time interval at which estimates of the LC50 were calculated was after 8 days when the LC50 was estimated to be 52.3 (24.8-110) and 63.7 (20.0-120) based on duplicate bioassays. After 21 days, we estimated the incipient LC50 for lindane and bluegill to be 29 (20-49) and 31 (20-49) µg/l. Duplicate continuous-flow bioassays were conducted at 25 ± 1°C with 2.0 gram fathead minnows and in both cases the 96-hour LC50 was >100 µg/l. After 11 days, the incipient LC50 for lindane and fathead minnows was estimated to be 62.5 (19.8-119) and 75.6 (13.4-143), based on duplicate bioassays. A single continuous-flow bioassay was conducted at 12 ± 1°C with 5.2 gram brook trout and indicated the 96-hour LC50 for lindane was 44.3 (32.7-60.1) µg/l. After 11 days we estimated the incipient LC50 for lindane to be 26 (19-39) µg/l. Based on these data, the highest nominal concentration selected for investigation during chronic exposure was 12 µg/l (bluegills), 25 µg/l (fathead minnows), and 20 µg/l (brook trout).

WATER CHEMISTRY

The results of the chemical analyses of water samples indicated that chemical characteristics varied minimally within any one chronic exposure. Therefore, only means and ranges for the various parameters are presented (Table 4). The results of gas-chromatographic analyses of water samples taken periodically during chronic exposures to lindane indicated that mean measured lindane concentrations closely approximated nominal concentrations (Table 5).

TABLE 4. MEAN AND RANGE OF MEASURED CONCENTRATIONS OF HARDNESS, ALKALINITY, ACIDITY, DISSOLVED OXYGEN AND pH FROM WATER SAMPLES TAKEN PERIODICALLY DURING CHRONIC EXPOSURE OF AQUATIC INVERTEBRATES AND FISHES TO LINDANE

Species	Hardness (mg/l)	Alkalinity (mg/l)	Acidity (mg/l)	Do (mg/l)	pH
Chironomus tentans					
Mean ± S.D.	37.0±3.6	33.0±1.9	5.3±1.1	6.9±1.1	-
Range	(32.0-43.0)	(28.0-39.0)	(4.0-6.8)	(5.6-8.3)	(6.3-7.6)
# of Samples	5	6	6	24	14
Daphnia magna					
Mean ± S.D.	38.0±2.4	32.0±2.6	5.7±0.8	8.6±1.2	-
Range	(33.0-39.0)	(26.0-44.0)	(4.7-6.8)	(6.8-8.9)	(6.1-7.3)
# of Samples	5	5	5	26	12
Gammarus fasciatus					
Mean ± S.D.	35.0±3.7	34.3±2.8	4.4±1.5	8.4±1.0	-
Range	(27.0-46.0)	(30.0-39.0)	(1.9-9.6)	(6.0-9.9)	(6.4-7.2)
# of Samples	42	42	42	162	12
Lepomis macrochirus					
Mean ± S.D.	37.4±3.8	33.2±4.5	4.4±1.7	6.4±1.0	-
Range	(29.0-42.9)	(24.8-38.5)	(1.9-7.7)	(3.3-8.5)	(6.4-7.4)
# of Samples	20	20	24	1856	30
Pimephales promelas					
Mean ± S.D.	40.2±3.1	35.3±2.4	4.7±1.7	7.2±1.0	-
Range	(32.0-46.2)	(31.0-39.5)	(2.9-9.6)	(3.9-9.7)	(6.5-7.4)
# of Samples	23	23	18	282	30
Salvelinus fontinalis					
Mean ± S.D.	37.0±4.4	32.2±4.4	4.8±1.6	8.2±0.8	-
Range	(31.7-44.0)	(25.6-38.4)	(2.9-7.7)	(4.5-11.1)	(6.5-7.3)
# of Samples	16	16	18	954	22

TABLE 5. NOMINAL AND MEASURED LINDANE CONCENTRATIONS (µg/l) IN WATER DURING CHRONIC EXPOSURE OF AQUATIC INVERTEBRATES AND FISHES TO LINDANE

Species and Nomin. Conc.	Measured Concentration Mean S.D.	Range	# of Samples
Chironomus tentans			
24	28.4 ± 4.9	16.0 - 42.0	7
12	10.8 ± 2.4	7.5 - 13.0	6
6	7.3 ± 1.2	2.6 - 8.7	6
3	5.0 ± 1.7	1.7 - 5.1	7
1.5	2.2 ± 0.8	0.6 - 2.4	6
Daphnia magna			
200	200 ± 70	109 - 296	20
100	120 ± 30	59 - 169	20
50	42 ± 10	19 - 112	20
25	19 ± 7	16 - 49	20
12	11 ± 6	6 - 27	20
Gammarus fasciatus			
20	17.7 ± 3.6	10.0 - 26.0	31
10	8.6 ± 2.1	4.0 - 12.0	32
5	4.3 ± 1.1	3.0 - 7.0	31
2.5	2.2 ± 0.5	1.2 - 3.4	32
1.2	1.2 ± 0.4	0.6 - 2.1	31
Lepomis macrochirus			
12	9.1 ± 4.1	3.9 - 20.0	86
6	4.4 ± 1.7	1.3 - 8.9	44
3	2.3 ± 1.0	1.0 - 6.0	58
1.5	1.1 ± 0.5	0.25- 2.0	45
0.8	0.6 ± 0.3	o.22- 2.0	55
Pimephales promelas			
25	23.0 ± 12.2	8.9 - 58.0	47
12	9.6 ± 5.2	2.8 - 32.0	28
6	5.6 ± 2.2	2.8 - 6.8	32
3	2.4 ± 1.0	1.1 - 5.5	30
1.5	1.3 ± 0.6	0.3 - 2.6	31
Salvelinus fontinalis			
20	16.6 ± 3.8	9.1 - 31.0	39
10	8.8 ± 2.3	6.6 - 16.0	24
5	4.1 ± 1.0	3.1 - 6.6	20
2.5	2.1 ± 0.7	1.2 - 3.9	23
1.2	1.0 ± 0.3	0.6 - 1.6	19

CHRONIC EXPOSURE

Chironomus tentans

Continuous exposure of the original Chironomus tentans eggs to a mean measured concentration of lindane as high as 28.4 µg/l had no significant effect on hatchability (Table 6). However, all first instar larvae from eggs exposed to 28.4 µg/l died within 3 days after hatching. Eggs continuously exposed to mean measured lindane concentrations of 10.8 and 7.3 µg/l generally hatched successfully, but virtually none of these larvae exceeded second instar development. Two larvae exposed to 7.3 µg/l did develop to fourth instar and pupate but did not successfully emerge as adults. Continuous exposure of chironomids to 5.0 µg/l lindane significantly delayed the pupation of larvae and the emergence of adults. Larvae exposed to 5.0 µg/l lindane completed pupation and emergence during days 28 to 30 of exposure. All remaining pupae from the control group, and those exposed to a mean measured lindane concentration of 2.2 µg/l, emerged between days 24 to 27 of exposure at which time the first generation exposure was terminated and the second generation exposure was initiated.

TABLE 6. SUMMARY OF THE EFFECT OF VARIOUS CONCENTRATIONS OF LINDANE ON HATCHABILITY, PUPATION AND EMERGENCE OF Chironomus tentans CONTINUOUSLY EXPOSED FOR TWO GENERATIONS

Mean measured lindane conc. (µg/l)	Rep[a]	Generation I			Generation II		
		% Hatch	Pupa	Adult	% Hatch	Pupa	Adult
Control	A	90	86	80	92	83	80
	B	94	91	86	84	76	71
2.2	A	92	90	87	93	89	85
	B	76	75	73	89	78	73
5.0	A	93	76	75	90	66	62
	B	86	90	80	91	38	36
7.3	A	55	2	0	-	-	-
	B	81	0	0	-	-	-
10.8	A	86	0	0	-	-	-
	B	47	0	0	-	-	-
28.4	A	83	0	0	-	-	-
	B	76	0	0	-	-	-

[a]One hundred eggs were initially exposed in each replicate.

Egg masses for the second generation exposure were obtained from treatments that contained first generation adults. Specimens which had emerged from the 5.0 and 2.2 µg/l treatments during the same period as had the controls (24 to 27 days) were taken, as were controls. The continuous exposure of second generation eggs to lindane concentrations as high as 5.0 µg/l did not significantly affect hatchability. Analysis of variance indicated that the number of second generation forms pupating and emerging was not significantly affected by continuous exposure to lindane. Also, there were no significant differences in the numbers of pupae, numbers of emerging adults produced, and mean time to emergence between the first generation and the second generation of Chironomus tentans continuously exposed to 2.2 or 5.0 µg/l lindane.

The results of continuous exposure to lindane through two generations of Chironomus tentans indicate that exposure to concentrations of 7.3 µg/l or more caused larval mortality, severe developmental retardations, and reduction of numbers of emerging adults. Continuous exposure to 5.0 µg/l lindane resulted in delayed emergence of adults. No significant effects of continuous exposure to 2.2 µg/l lindane was observed. Based on the effect of lindane on the development, survival and emergence of Chironomus tentans, the estimated maximum acceptable toxicant concentration for this species is >2.2 and <5.0 µg/l.

Daphnia magna

Statistical analysis of data on survival of Daphnia continuously exposed to lindane for 64 days indicates significant differences due to treatment. Through the first two generations, continuous exposure to mean measured concentrations of 210, 120 and 42 µg/l lindane significantly reduced survival of daphnids (Table 7). This effect on survival was cumulative, with survival of second generation daphnids being significantly less than that of the first generation. Survival of second generation Daphnia continuously exposed to a mean measured lindane concentration of 11 µg/l was significantly lower than controls. However, this does not appear to be toxicant-related as survival of second generation daphnids exposed to a mean measured lindane concentration of 19 µg/l was comparable to that of controls. Survival of third generation Daphnia continuously exposed to 19 µg/l lindane was significantly less than controls, indicating again that the effects on daphnids of continuous exposure to lindane are cumulative. An untimely mechanical malfunction of the exposure system resulting in the death of most of the third generation

TABLE 7. MEAN[a] PERCENT SURVIVAL OF Daphnia magna CONTINUOUSLY EXPOSED TO LINDANE FOR 64 DAYS

Mean measured conc. (µg/l)	Generation[b] I			Generation[b] II			Generation[b] III		
	Day			Day			Day		
	8	15	70	29	36	43	50	57	64
Control	90	73	70	100	93	86	93	93	86
11	90	83	78	100	48	45	20[c]	20	15
19	95	90	78	97	80	70	60	60	45
42	73	58	45	75	58	32	38	32	27
120	90	85	58	20	20	13	22	15	10
210	83	75	38	25	12	0	0	0	0

[a]Each value represents the mean of four replicates.

[b]Duration of exposure for generations, I, II, and III were days 1-22, 22-43, 43-64, respectively.

[c]Test containers overflowed on day 46 resulting in the death of most test organisms at this concentration.

Daphnia exposed to 11 µg/l lindane precludes a valid assessment of this exposure level during this period.

Continuous exposure to 210 µg/l lindane virtually prevented production of young among the surviving Daphnia during the second generation exposure (Table 8). Statistical analysis of data concerning production of young during the first two generations of exposure to lindane indicates that exposure to lindane concentrations of 120 and 42 µg/l significantly reduced the number of young produced per female. Assessment of the effect of the toxicant on production of young during the third generation exposure is hindered by the poor production of young observed in the control group. However, it is important to note that the number of young produced per female among Daphnia continuously exposed to 19 µg/l lindane was significantly less that of Daphnia exposed to 11 µg/l, suggesting a toxicant-related effect. It is also important to note that the number of young produced per female among third generation daphnids exposed to 11 µg/l lindane was the highest observed during the experiment suggesting that toxicant-induced inimical effects did not occur at this level of exposure.

Based on the observed effects of lindane on survival and reproduction of Daphnia magna continuously exposed to the toxicant through three generations of development, the estimated maximum acceptable toxicant concentration for this species is >11 and <19 µg/l.

TABLE 8. MEAN[a] PRODUCTION OF YOUNG PER FEMALE *Daphnia magna* CONTINUOUSLY EXPOSED TO LINDANE FOR 64 DAYS

Mean Measured conc. (µg/l)	Generation[b] I		Generation[b] II		Generation[b] III	
	Day		Day		Day	
	15	22	36	43	57	64
Control	4.6	29.7	35.5	23.4	6.5	8.2
11	4.8	27.0	28.1	29.4	37.5	35.3
19	8.6	22.0	25.5	22.7	12.7	7.7
42	6.6	17.0	13.7	14.6	20.7	7.2
120	5.7	15.7	16.0	12.2	9.3	6.4
210	6.2	9.8	0.5	0	0	0

[a]Each value represents the mean of four replicates.

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, 43-64, respectively.

Gammarus fasciatus

Statistical analysis of data on survival of *Gammarus fasciatus* indicated significant differences due to treatments (Table 9). Continuous exposure to mean measured lindane concentrations of 17.7 and 8.6 µg/l for 30 days significantly reduced survival of gammarids (Table 9). No effect on survival of gammarids continuously exposed to a mean measured lindane concentration of 4.3 µg/l for 120 days was observed, when compared to controls.

Pairing of males and females was first observed on days 35 and 36 among adult gammarids in the controls and in the three lowest treatments (4.3, 2.2 and 1.2 µg/l). No significant pairing of adult gammarids exposed to 17.7 µg/l lindane was observed. Two gravid females were observed among this group but no young were shed. Some pairing among gammarids exposed to 8.6 µg/l lindane was observed on day 40. However, none of the young that were subsequently shed survived exposure to this concentration of lindane. Mating pairs of *Gammarus* from the controls and from the 4.3, 2.2 and 1.2 mg/l lindane treatments began to shed young between days 50 to 60 of exposure (6 to 13 days after isolating the females). In all of these groups, approximately half of all gravid females failed to shed young.

Although there was virtually no production of young in one replicate of gammarids exposed to 4.3 or 8.6 µg/l lindane, production in the other replicate was comparable to that of the two lower treatments (2.2 and 1.2 µg/l) and of controls, suggesting that the observed failure was not toxicant-related.

TABLE 9. SURVIVAL AND REPRODUCTIVE SUCCESS OF *Gammarus fasciatus* EXPOSED TO LINDANE FOR 17 WEEKS

	Mean Measured Lindane Concentration (µg/l)											
	17.7		8.6		4.3		2.2		1.2		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
Adult Survival (%)												
Day 30	60	60	73	73	90	90	87	83	93	93	93	83
60	27	27	43	47	47	77	57	63	70	87	83	77
90	7	10	23	17	30	30	23	27	27	73	30	17
120	0	0	3	0	3	10	6	6	10	16	10	16
Total # Young	-	-	50	1	1	38	41	39	54	47	38	48
# young continued in treatment	-	-	50	1	1	38	25	23	54	40	38	48
Survival after 30 days (%)	-	-	0	0	0	35	66	33	65	82	60	4

Data on the survival of second generation gammarids beyond 30 days are extremely variable and preclude statistical evaluation and interpretation. Based on the effects of lindane on survival and reproduction of Gammarus fasciatus, the estimated maximum acceptable toxicant concentration of lindane for this species is >4.3 and <8.6 µg/l.

Lepomis macrochirus

Survival and growth of bluegills exposed to lindane for 6 and 18 months were similar for all treatments (Table 10). Spawning activity occurred between test days 592 and 716, with most activity observed in tanks receiving 2.3 µg/l of lindane. Spawning activity was too sporadic to be conclusive and results of spawning (number of eggs, hatchability and fry survival) were not analyzed statistically. An attempt was made to induce further spawning from bluegills by administering interperitoneal injections of carp pituitary extract on test days 714 and 715. All fish were injected with 0.5-1.0 cc of a 200 µg/100 ml solution of carp pituitary in physiological saline. The injections failed to induce the desired increase in spawning and may have been administered too late in the spawning period to be effective. Parental fish were sacrificed on test day 735 and samples of muscle were retained for residue analysis.

Results of egg hatchability and survival and growth of bluegill fry are presented (Table 11). Due to the lack of spawning of control fish, it is difficult to assess possible toxicant-related effects on these parameters. Survival of bluegill fry after 30 days was higher in tanks receiving 2.3 µg/liter of lindane than in those receiving 4.4 and 1.1 µg/liter.

Survival during this period appeared to be related to an early acceptance of available food rather than lindane concentration. This observation is supported by the fact that survival did not greatly change during the last two months of exposure when feeding habits became well established and food was more readily accepted. Total lengths at 30, 60 and 90 days did not appreciably vary for bluegill fry exposed to 4.4, 2.3 and 1.1 µg/l of lindane.

Based on the data obtained from continuous exposure, it appears that the maximum acceptable toxicant concentration is greater than the highest measured lindane concentration (9.1 µg/l) to which bluegills were exposed during the test. Because ten percent mortality was observed among bluegills continuously exposed to 12.5 µg/l lindane for 23 days in a continuous-flow acute bioassay, the maximum acceptable toxicant concentration of lindane for this fish is estimated to be between >9.1 and <12.5 µg/l.

TABLE 10. SURVIVAL AND GROWTH DURING 6 AND 18 MONTHS, AND RESULTS OF SPAWNING ACTIVITY OF BLUEGILL (Lepomis macrochirus) CONTINUOUSLY EXPOSED TO LINDANE

Item	Measured lindane concentration (ug/liter)											
	9.1		4.4		2.3		1.1		0.6		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
6 MONTHS												
Survival (%)[a]	100	100	75	100	100	100	80	90	90	100	85	100
Total Length (mm)[b]	122	122	122	122	119	121	123	120	116	119	128	127
Total weight (g)[c]	33	33	33	34	31	33	34	31	26	29	40	38
18 MONTHS												
Survival (%)[d]	80	90	70	90	80	70	90	80	90	100	90	80
♂/♀	2/6	5/4	4/3	2/7	4/4	3/4	4/5	3/5	4/5	5/5	5/4	2/6
Total length (mm)												
♂	200	177	186	213	193	187	194	195	184	177	189	195
♀	161	516	158	166	153	155	152	153	151	147	153	157
Total weight (g)												
♂	157	105	130	194	145	134	151	147	141	133	123	153
♀	75	64	78	84	65	73	63	51	59	58	60	68
#♀ spawning	0	1	3	0	4	4	1	4	3	0	0	0
Eggs/♀	-	4523	9742	-	11277	14745	2915	8032	6584	-	-	-
Spawns/♀	-	2	2	-	3	4	1	2	1	-	-	-

[a]Survival based on 20 fish/tank.

[b]Mean initial length/fish 93 mm.

[c]Mean initial weight/fish 11g.

[d]Survival after thinning based on 10 fish/tank.

TABLE 11. HATCHABILITY, SURVIVAL AND GROWTH OF BLUEGILL (Lepomis macrochirus) FRY EXPOSED TO LINDANE

Item	Measured Lindane Concentration (µg/l)			
	4.4	2.3	1.1	0.6
Hatchability (%)	70	65	88	90
# Egg groups[a]	(2)	(11)	(1)	(3)
# fry groups[b]	1	4	1	1
30 days survival (%)	16	60	12	0
60 days survival (%)	14	59	12	0
90 days survival (%)	14	59	12	0
Mean total length (mm)				
30 days	19 ± 1.8	19 ± 2.3	24 ± 1.0	-
60 days	31 ± 4.0	30 ± 3.8	28 ± 3.5	-
90 days	39 ± 3.6	39 ± 6.1	30 ± 5.3	-

[a] Each group contained 200 eggs.

[b] Each fry group contained 30 one day old fry.

Pimephales promelas

Survival of fathead minnows exposed to a mean measured lindane concentration of 23.5 µg/l for 60 days appeared lower than for all other treatments and the controls (Table 12). However, variability in the data precluded ascribing statistical significance to this 60-day observation.

Statistical analysis of the survival after 43 weeks exposure to 23.5 µg/l substantiated this trend and showed a significant decrease in survival of fathead minnows exposed to this lindane concentration as compared with control groups and lower treatments. Spawning of fathead minnows in all lindane treatments and the controls occurred between test days 124 and 271. For unknown reason(s), the number of mature females and number of eggs produced per female were significantly lower in controls than in all groups exposed to lindane (Table 13). We do not believe that this observation is indicative of some positive effect of lindane on reproduction since the number of mature females, the number of spawnings per female, the number of eggs per mature female, and the number of eggs per spawn observed in groups exposed to lindane are similar to those observed for fathead minnows from control groups and from unaffected treatments in other chronic studies. Conversely, the same parameters for controls in this

TABLE 12. SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) DURING 30 DAYS, 60 DAYS, AND 43 WEEKS CONTINUOUS EXPOSURE TO LINDANE

Item	LINDANE (µg/l)											
	23.5		9.1		5.6		2.4		1.4		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
30 DAYS												
Survival (%)	80	90	94	96	80	86	92	98	74	82	86	84
Total length (mm)	11	11	10	10	12	11	11	11	11	10	11	10
(S.D.)[a]	(1.4)	(1.8)	(1.8)	(1.7)	(1.8)	(1.8)	(1.7)	(1.8)	(2.6)	(1.6)	(2.5)	(2.0)
60 DAYS												
Survival (%)[b]	50	58	86	76	76	54	86	78	56	60	84	62
Total length (mm)	19	20	18	19	20	20	16	22	15	18	14	17
(S.D.)	(3.9)	(4.0)	(5.7)	(3.5)	(4.7)	(5.7)	(4.4)	(4.6)	(5.4)	(5.8)	(3.9)	(6.4)
43 WEEKS												
Survival (%)[c]	73	73	93	93	93	93	100	93	100	87	93	93
♂/♀	4/5	4/7	3/10	3/7	3/6	3/7	6/7	4/8	4/7	3/9	3/7	4/6
Extra ♂ removed	2	0	1	4	5	4	2	2	4	1	4	4
Total length (mm)												
♂	72	71	65	68	66	70	66	70	66	69	59	64
♀	55	57	55	55	57	56	55	55	55	57	49	55
Total weight (g)												
♂	3.3	3.3	2.7	3.0	2.7	3.0	2.7	3.0	2.8	3.0	2.0	2.0
♀	1.2	1.6	1.3	1.4	1.5	1.4	1.1	1.3	1.3	1.6	1.3	1.3

[a] Standard Deviation.

[b] Based on 40 fish/tank.

[c] Based on 15 fish/tank after thinning at 60 days.

TABLE 13. SEXUAL DEVELOPMENT, SPAWNING, HATCHABILITY OF EGGS, AND SURVIVAL AND GROWTH OF OFFSPRING AFTER 30 AND 60 DAYS, FOR FATHEAD MINNOW (*Pimephales promelas*) CONTINUOUSLY EXPOSED TO LINDANE

Item	Mean Measured Lindane Concentration (µg/liter)											
	23.4		9.1		5.6		2.4		1.4		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
# mature ♀	5	7	8	7	6	6	7	8	5	8	2	3
Spawning/♀	11	9	8	8	14	12	8	4	8	6	4	2
Eggs/♀	1494	1057	907	751	1504	1120	871	387	783	582	147	145
Eggs/spawning	136	117	113	94	107	93	109	97	98	198	37	73
Percent Hatch[b]	86	92	88	90	89	87	88	82	91	82	87	94
(N)	(27)	(29)	(31)	(20)	(32)	(31)	(32)	(16)	(18)	(8)	(4)	(5)
30 DAYS												
Survival (%)	89.0	84.0	72.0	45.0	71.0	75.0	71.0	45.0	88.0	42.0	78.0	55.0
Total length (mm)	11.0	10.0	11.0	13.0	12.0	12.0	12.0	12.0	11.0	12.0	10.0	10.0
(S.D.)[a]	(2.4)	(2.2)	(2.2)	(2.6)	(2.9)	(2.1)	(2.9)	(1.8)	(2.4)	(3.2)	(2.7)	(2.0)
60 DAYS												
Survival (%)	83.0	83.0	70.0	45.0	67.0	70.0	70.0	45.0	84.0	33.0	75.0	53.0
Total length (mm)	18.0	19.0	21.0	20.0	19.0	20.0	21.0	21.0	18.0	20.0	17.0	21.0
(S.D.)	(4.8)	(3.7)	(4.6)	(3.7)	(4.2)	(4.0)	(4.5)	(3.4)	(4.5)	(5.3)	(4.9)	(5.9)
# fry groups[c]	2	2	2	2	4	4	4	2	4	2	1	1

[a] Standard Deviation.

[b] Hatchability samples contained 50 eggs.

[c] Each fry group contained 40 one day old fry.

study were severely depressed. We have no basis on which to speculate whether this observation is a random occurrence or what cause-effect relationship might be applicable. Parental fish were sacrificed on test day 304 after all spawning had ceased for 3 weeks and samples of the edible portion (eviscerated carcass) retained for residue analysis.

Statistical analysis indicated no significant differences in hatchability and percent survival and growth of F_1 fathead minnow fry after 30 and 60 days continuous exposure to lindane. Based on these data derived from the continuous exposure of fathead minnows to various concentrations of lindane for 52 weeks, the estimated maximum acceptable toxicant concentration for this species is >9.1 and <23.5 µg/l.

Salvelinus fontinalis

Continuous exposure for 261 days to mean measured lindane concentrations as high as 16.6 µg/l had no significant effect on survival of brook trout. Although continuous exposure to lindane did not significantly effect growth of trout during the first 90 and 136 days exposure, continuous exposure of fish for 261 days to a mean measured lindane concentration of 16.6 µg/l significantly reduced the weight and total length of brook trout when compared to the controls and other exposed groups (Table 14).

Spawning activity of yearling brook trout in all experimental units occurred between days 189 and 242 of exposure. The failure of most control fish to spawn their eggs and the subsequent inconsistencies in data related to spawning activity preclude valid conclusions regarding statistical significance of these data (Table 15). Certainly, however, it would appear that continuous exposure to 16.6 µg/l lindane reduced the percent of eggs developing neural keel, and reduced the percent of eggs successfully hatching, below those observed for all other treatments. Also, we observed that brook trout exposed to 16.6 µg/l lindane usually did not spawn on the substrate provided (as did fish in all other groups) but frequently released eggs randomly throughout the experimental chamber making fertilization by the male virtually impossible. Adult brook trout were sacrificed after 261 days of exposure when no spawning had been observed for 21 days, and samples of muscle tissue taken for residue analysis.

No significant effects of exposure to lindane on survival and growth of second generation brook trout were observed (Table 16). During the first 30 days of exposure (prior to swim-up), survival of fry exposed to 16.6 µg/l lindane was 94.5% as compared to 97.0% among unexposed fish. During the remaining 60 days observation, survival was generally

TABLE 14. GROWTH OF YEARLING BROOK TROUT[a] (Salvelinus fontinalis) DURING 261 DAYS CONTINUOUS EXPOSURE TO LINDANE

Item	Mean Measured Lindane Concentration (ug/liter)											
	16.6		8.8		4.1		2.1		1.0		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
Mean length (mm)	251	255	265	264	263	270	272	274	270	259	281	277
(S.D.)[b]	(26)	(10)	(21)	(11)	(23)	(15)	(23)	(12)	(16)	(13)	(11)	(19)
Mean weight (g)	172	175	219	187	203	212	229	219	229	194	255	255
(S.D.)	(47)	(20)	(72)	(28)	(54)	(64)	(67)	(36)	(58)	(42)	(53)	(72)

[a]Initial mean total length, 199 mm, initial mean total weight, 73 g; initial number of fish/tank, 15.

[b]Standard Deviation.

TABLE 15. RESULTS OF SPAWNING ACTIVITY OF YEARLING BROOK TROUT (Salvelinus fontinalis) DURING CONTINUOUS EXPOSURE TO LINDANE

Item	Mean Measured Lindane Concentration (ug/liter)											
	16.6		8.8		4.1		2.1		1.1		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
No. ♂/♀	1/3	2/4	2/4	3/3	2/4	3/3	2/4	2/4	2/4	2/4	2/4	3/3
No. ♀ spawning	3	4	4	3	4	3	4	4	3	4	3[a]	3[a]
No spawns/♀	3	3	1	4	3	3	3	3	2	3	2	2
Total # eggs spawned	1145	1461	1210	1731	2630	2355	2260	2188	1747	2195	425	1015
#Eggs spawned/♀	382	365	302	577	658	732	565	547	437	549	106	338
Neural Keel developed (%)	0	28	50	37	61	78	80	76	76	31	0	72
Incubation time (degree days)	-	405	387	396	423	414	423	404	441	432	-	369
Mean hatchability (%)	-	28	76	92	65	72	97	51	86	68	-	25
(N)[b]	0	(1)	(1)	(2)	(3)	(5)	(4)	(4)	(1)	(1)	-	(2)

[a] Unlike females from all other experimental units, females in this unit were not spent at termination of exposure but were observed to contain mature eggs.

[b] Indicated the number of groups of 50 eggs in which at least half successfully developed a neural keel.

TABLE 16. SURVIVAL AND GROWTH OF SECOND GENERATION BROOK TROUT (Salvelinus fontinalis) DURING THE FIRST 90 DAYS DEVELOPMENT OF FRY CONTINUOUSLY EXPOSED TO LINDANE

Item	Mean Measured Lindane Concentration (µg/liter)											
	16.6		8.8		4.1		2.1		1.1		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
No. Groups	-	1	2	2	2	2	2	2	2	2	2[a]	2[a]
Survival (%)												
30 Days	-	93	96	94	92	72	90	100	59	79	94	100
60 Days	-	7	23	4	32	0	50	46	2	25	54	48
90 Days	-	7	23	4	32	0	50	46	2	25	54	48
Mean Length (mm)												
30 Days	-	20	20	20	20	19	21	21	19	21	22	21
60 Days	-	20	21	21	22	20	24	23	21	22	29	27
90 Days	-	21	22	21	23	-	27	28	25	26	42	33

[a]These groups were obtained from a local hatchery and were of the same age as the remaining groups.

poor and response could not be correlated with treatments. Except for the unexposed fish (obtained from a local hatchery) growth of second generation fry during the final 60 days observation was very poor and may or may not be toxicant-related.

Based on the available evidence, derived from continuous exposure of brook trout to lindane for 261 days, relating to pesticide-induced reduction of growth, and apparent effects on percent fertilization and hatchability, we estimate the maximum acceptable toxicant concentration of lindane for brook trout is >8.8 and <16.6 µg/l.

RESIDUE ANALYSIS

Three samples of muscle tissue from terminated adult bluegills and brook trout were analyzed to determine lindane residues in fish exposed to each concentration of lindane. The small size of fathead minnows prompted the use of 3 samples of pooled eviscerated carcasses of terminated adults exposed to each lindane concentration. Results of these analyses (Table 17) clearly show that the amount of lindane residue accumulated is directly proportional to the level of exposure and that this response is essentially linear over the range of concentrations tested.

Although the bioconcentration factors for brook trout and bluegills are similar, those for fathead minnows appear to be an order of magnitude higher. This may reflect the possible inclusion of tissue other than muscle with the fathead minnow carcass (e.g. kidney). Alternatively, these observations may be indicative of species differences in the tendency of fishes to accumulate and retain chemical residues. It is interesting to note that the control fish of each species appear to have concentrated the trace amount of lindane in the diluent water by a factor similar to that observed for other experimental groups.

CALCULATION OF APPLICATION FACTORS

A summary of the estimated LC50 value, the maximum acceptable toxicant concentration, and the application factor derived therefore, for lindane and all species studied is presented (Table 18). The MATC for each species except bluegills is, by definition (Mount and Stephan, 1967), an undetermined value between the highest mean measured concentration having no significant effect and the lowest mean measured concentration significantly affecting the organism during chronic exposure. The MATC for bluegills is estimated between the highest mean measured concentration applied during chronic exposure and the minimum mean measured concentration which produced

TABLE 17. MEAN MEASURED LINDANE CONCENTRATION IN WATER (µg/l) AND IN THE MUSCLE (µg/kg) OF BLUEGILL (Lepomis macrochirus) AND BROOK TROUT (Salvelinus fontinalis) AND IN THE EVISCERATED CARCASS (µg/kg) OF FATHEAD MINNOWS (Pimephales promelas) CONTINUOUSLY EXPOSED TO LINDANE

Item	Treatment					
	1	2	3	4	5	Control
Bluegills						
735 days-water	9.1	4.4	2.3	1.1	0.6	0.05
Muscle	297±38	196±160	62±43	25±4	16±14	2.8±1.6
Bioconcentration factor (X)	33	45	27	23	27	56
Brook trout						
261 days-water	16.6	8.8	4.1	2.1	1.0	0.05
Muscle	1200±283	770±113	255±7	227±86	51±36	2±1
Bioconcentra-factor (X)	72	88	62	108	51	40
Fathead minnows						
304 days-water	23.5	9.1	5.6	2.4	1.4	0.05
carcass	9533±1101	6133±2359	2867±551	1067±515	537±165	27±6
Bioconcentration factor (X)	406	674	512	445	284	540

TABLE 18. SUMMARY OF CONCENTRATIONS OF LINDANE (µg/l) PRODUCING ACUTE AND CHRONIC TOXICITY TO AQUATIC SPECIES, AND CALCULATED APPLICATION FACTORS DESCRIBING THE RELATIONSHIP BETWEEN ACUTE AND CHRONIC TOXICITY

Species	Common	LC50[a]	MATC limits	Limits on application factor
Chironomus tentans	midge	207 (157-273)[b]	>2.2<5.0	0.01&0.02
Daphnia magna	water flea	485 (391-623)	>11.0<19.0	0.02&0.03
Gammarus fasciatus	scud	39 (27-56)	>4.3<8.6	0.11&0.22
Lepomis macrochirus	bluegill	30 (20-49)	>9.1<12.5	0.30&0.42
Pimephales promelas	fathead minnow	69 (24-112)	>9.1<23.5	0.13&0.34
Salvelinus fontinalis	brook trout	26 (19-39)	>8.8<16.6	0.34&0.64

[a] 48-hour LC50 for invertebrates, incipient LC50 for fishes.

[b] 95% confidence interval.

significant mortality during acute exposure. Application factors describing the relationship between the acute and chronic toxicity of lindane for the species studied are calculated using the MATC and the 48-hour LC50 for invertebrates. Similar application factors are calculated for fishes utilizing the limits on the MATC and the incipient LC50 which Eaton (1970) suggests to be a better measure of acute toxicity for this type of calculation.

Application factors are similar for two of the three invertebrate species tested. Application factors for the fishes are remarkably similar. However, the factors calculated for two of the invertebrates are an order of magnitude smaller than those calculated for the fishes.

SECTION VI

DISCUSSION

The estimated 48-hour LC50 value of 39 µg/l determined for Gammarus fasciatus is lower than the 48-hour EC50 of 88 (57-136) µg/l which Cope (1966) obtained with G. lacustris. This discrepancy may be attributed to either species differences in susceptibility, or to differences in age of the test animals between the two studies, or both. The estimated 48-hour LC50 of 485 µg/l which we determined for Daphnia magna is essentially equivalent to the previously reported value of 460 ug/l (FWPCA, 1968). No comparable toxicity data are available for chironomids and lindane. However, Karnak and Collins (1974) reported that the LC50 values for eleven insecticides to third and fourth instar larvae of Chironomus tentans were generally within the range of 1-10 µg/l. These data suggest either that first instar midge larvae are generally more resistant to insecticide poisoning than third and fourth instar forms, or that lindane is generally 1-2 orders of magnitude less toxic to this species than the insecticides they investigated, or a combination of both.

As might be expected, the incipient LC50 values for lindane to bluegills (30 µg/l) and fathead minnows (69 µg/l) determined in flowing bioassays were slightly lower than the 96-hour LC50 values reported by Macek and McAllister (1970) for bluegills (68 µg/l) and fathead minnows (87 µg/l) bioassayed under static conditions. The incipient LC50 of lindane to brook trout (26 µg/l) was essentially identical to 96-hour LC50 reported by these authors for rainbow trout (27 µg/l).

The results of chronic exposure of the three invertebrate species to lindane indicate that daphnids are the least susceptible of the invertebrates tested, as they were during acute toxicity bioassays. The midges and scuds were similar in their susceptibility to chronic exposure to lindane, and were the most sensitive of all the species tested. The chironomids and daphnids exhibited the greatest differences between susceptibility to acute lindane exposure and susceptibility to chronic lindane exposure. One can speculate that this may be related to the developmental physiology of these organisms. For example, the midges experience four life forms (egg, larvae (four instars), pupae and adult) which are distinctively different in their biochemical and physiological processes. Conceivably the chronic exposure of Chironomus to chemicals incorporates four distinct exposure phenomena which individually and/or cumulatively are

responsible for increased chemical susceptibility. These phenomena may also be cause to select a species like the midge for evaluating the hazard to aquatic organisms associated with the occurrence of chemicals in aquatic ecosystems.

Except for Gammarus, little previous information is available to provide a basis for making generalizations about the relative susceptibility of fishes and invertebrates to chronic exposure to chemicals in water. However, much of the previously reported information leads one to the conclusion that invertebrates generally are more susceptible to chronic exposure than fishes. Pickering and Thatcher (1970) reported the MATC of LAS for fathead minnows >0.63<1.2 mg/l, while Arthur (1970) reported the MATC of LAS for Gammarus pseudolimnaeus to be >0.2<0.4 indicating this invertebrate species may be ca 3 times more susceptible to LAS than the minnow.

Mount and Stephan (1969) reported the MATC of copper in soft water for fathead minnows was >10.6<18.4 µg/l, while Arthur and Leonard (1970) reported the MATC of copper in soft water for G. pseudolimnaeus was >4.6<8.0 indicating again that the invertebrate species may be at least 2 times more susceptible than the minnow. More recently, Arthur et al., (1973) has reported that gammardis may be 3 times more susceptible to chronic exposure to NTA than were fathead minnows. Certainly, all of our results of chronic exposure of invertebrates to lindane support a hypothesis that invertebrates are generally more susceptible to chronic exposure to many chemicals than fishes and suggests they may be very useful in evaluating chronic toxicity of chemicals to aquatic organisms.

Only one similar chronic toxicity study with bluegills has been previously described (Eaton, 1970). In comparing the data from our study with those reported by Eaton, it appears that the bluegills in our study were somewhat larger and older. As a result, where spawning occurred, we observed more spawns per female and greater egg production per female than occurred in the previous work. It is extremely unfortunate that the factors influential in stimulating or inhibiting spawning activity in our study could not be adequately identified. The percent hatch of bluegill eggs which we observed (65-90%) was comparable to that observed by Eaton (ca 72%). We experienced difficulty in maintaining and feeding bluegill fry during the initial 90 day developmental period. We attribute this primarily to an inability to provide adequate amounts of suitable food forms during the various stages of fry development. Eaton (1970) experienced the same difficulties and suggested that lack of suitable food was the primary factor responsible for poor fry

survival. Before additional significant effort is expended to investigate chronic toxicity of chemicals to bluegills, definitive information on what are suitable food sources for developing bluegill fry, and how adequate numbers of these can be effectively made available to the fry, must be developed.

The results of our investigation of the chronic toxicity of lindane to fathead minnows, though not without its problems, reinforces our opinion that this species provides the best opportunity for evaluating chronic toxicity chemicals to fishes. Certainly more work has been reported with this species than any other (Mount, 1968; Brungs, 1969; Mount and Stephan, 1969; Hermanutz et al., 1973) and procedures for utilizing this species in long-term laboratory assays are becoming well defined. Except for the control groups, the results of our fathead minnow chronic study in terms of performance of the test organisms are good and indicate again that subtle long-term effects of chemicals on fishes can be evaluated under controlled laboratory conditions using this species.

The number of spawnings per female, the number of eggs per spawn, the percent hatchability, and the survival of fry during the first 60 days development in the lindane study were as good or better than that generally observed in previous studies. In view of these facts, and the fact that the fathead minnow procedure represents the only "true chronic bioassay" of the three species tested (i.e. at least one complete life cycle) we feel strongly that fathead minnows should be the fish species of choice until methods suitable for using other species are sufficiently defined.

We feel the brook trout offers significant potential for use in "partial chronic bioassays". Although there were inconsistencies in the spawning activity from group to group, generally the numbers of fish participating in spawning and the number of eggs spawned were adequate. Except for those situations where toxicant-related effects on spawning were indicated, the percent fertilization of eggs, percent hatch and survival through the first 30 days (to swim-up) were generally good. We feel that significant valid information can be generated utilizing this species and these procedures. However, certain areas of the methods need refinement. One suggestion we have is that separate systems, free from the effect of light, be provided for the egg incubation. Leitritz (1959) suggested that both trout and salmon eggs are sensitive to direct light. In our experiment the light intensity at the water surface varied from 18

foot-candles (fc) in most tanks to 35 fc in four tanks randomly positioned under the center of the fluorescent lights. The tanks receiving the highest light intensity were both control units, duplicate A of those fish exposed to 1.0 µg/l, and duplicate B of those fish exposed to 4.1 ug/l lindane. These groups were among those exhibiting the lowest percent hatchability of eggs, and percent survival of fry (Tables 15 and 16).

We also suspect that the decreased survival of fry observed in all groups during the period 30-90 days after hatch may be related to the stress associated with handling fry at 30 and 60 days to count and measure those organisms. We suggest that a single measurement after 60 days exposure would provide the same useful information regarding potential effects of chemicals on second generation trout yet would minimize possible effects of handling on survival. We have recently conducted several similar studies with brook trout eggs and fry and achieved excellent success hatching and fry survival by shielding the developing eggs from light and minimizing handling of fry.

We have suggested that continuous exposure to 16.6 µg/l lindane for 261 days produced an inimical effect on the spawning behavior of brook trout resulting in ineffective fertilization of spawned eggs. Boyd (1964) reported that 15% of mosquito fish aborted their young after surviving lindane concentrations which produced 10-40% mortality among fish.

The accumulation of lindane residues in the muscle of bluegills exposed for 735 days found to be 23 to 56 times the mean lindane concentration in the water in which these fish were exposed. Gakstatter and Weiss (1967) found that 6-9 g bluegill exposed for 19 hours to 30 µg/l lindane accumulated whole body lindane residues of 1500 µg/kg, a bioconcentration factor of 50X. These data suggest that ca 30-50X probably represents the equilibrium bioconcentration factor of lindane by bluegill and that this equilibrium is rapidly established and maintained throughout exposure. Although we did not investigate the rate of residue elimination, Gakstatter and Weiss reported that most of the lindane was eliminated within two days after transfer to uncontaminated water.

During the last two years we have had the opportunity to investigate the bioconcentration of some 40 pesticides by bluegills during a minimum of 30 days continuous exposure (Bionomics, unpublished data). With all but three of the pesticides studied, we observed bioconcentration factors

of at least 10X, and with more than half of those studied we observed bioconcentration factors >50X. These data suggest that bluegills do not bioconcentrate lindane to any greater extent than they do most pesticides. Certainly, this bioconcentration factor does not appear as significant as those reported for chemicals which have recently been the cause of great concern among regulatory agencies and environmentalists. For example, Macek and Korn (1970) reported brook trout continuously exposed to 3 ng/l DDT for 120 days concentrated the chemical ca 8500X. Dr. R. Reinert U.S.D.I., Great Lakes Research Lab., Ann Arbor, Michigan has found that rainbow trout bioconcentrate methylmercury by as mush as 8000X (personal communication). Hansen et al. (1971) reported marine fish exposed to polychlorinated biphenyls concentrated the material 30,000X.

Mount and Stephan (1967) have proposed the utilization of application factors to estimate chronic toxicity of chemicals to fish based on acute toxicity data, and considerable information based on chronic exposure of a variety of fishes to pesticides and heavy metals support this hypothesis. Certainly, the results of these investigations with lindane provide evidence to substantiate the merits of this hypothesis. The similar limits of the application factor for each of the fishes tested is indeed remarkable.

Previous information has suggested that application factors for pesticides are generally 0.1 or less. The only materials for which application factors have been estimated to be on the order of 0.25 or greater are LAS and NTA, materials which are either relatively non-toxic to aquatic organisms, readily biodegradable, or both. In view of the previously reported application factors for chemicals and fishes it was surprising, to say the least, to determine that the application factors for lindane were so large (Table 19).

TABLE 19. SUMMARY OF APPLICATION FACTORS REPORTED FOR CHEMICALS TO VARIOUS SPECIES OF FISH

Chemical	Species	Limits on application factors	Source
Copper	trout	.100-.170	McKim and Benoit, 1971
Copper	minnow	.035-.076	Mount, 1968
Copper	minnow	.120-.220	Mount & Stephan, 1969
Zinc	minnow	.003-.020	Brungs, 1969
Cadmium	minnow	.005-.008	Pickering and Gast, 1972
LAS	minnow	.145-.276	Pickering & Thatcher, 1970
2,4-D(BEE)	bluegill	.053-.268	Mount & Stephan, 1967
Malathion	minnow	.022-.066	Mount & Stephan, 1967
Malathion	bluegill	.043-.090	Eaton, 1970
NTA	minnow	>.500	Arthur et al., 1973

SECTION VII

REFERENCES

American Public Health Association 1971. Standard Methods for the Examination of Water and Wastewater, 13th ed. Am. Public Health Assoc., New York. 874 p.

Arthur, J. W. 1970. Chronic effects of linear alkylate sulfonate detergent on Gammarus pseudolimnaeus, Campeloma decisum and Physia integra. Water Research 4:251-257.

Arthur, J. W., A. E. Lemke, V. R. Mattson, and B. J. Halligan 1973. Toxicity of sodium nitrilotriacetate (NTA) to the fathead minnow and an amphipod in soft water. Water Research 8:187-193.

Arthur, J. W. and E. N. Leonard 1970. Effects of copper on Gammarus pseudolimnaeus, Physa integra and Campeloma decisum in soft water. J. Fish. Res. Bd. Canada 27: 1277-1283.

Benoit, D. A. 1974. Artificial laboratory spawning substrate for brook trout (Salvelinus fontinalis, Mitchill). Trans. Am. Fish. Soc. 103:144-145.

Biesinger, K. E., and G. M. Christensen 1972. Effects of various metals on survival, growth, reproduction, and metabolism of Daphnia magna. J. Fish. Res. Bd. Canada 29:1691-1700.

Bioassay Committee 1971a. Recommended bioassay procedures for fathead minnow (Pimephales promelas, Rafinesque) chronic tests. National Water Quality Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota. 13 p.

Bioassay Committee 1971b. Recommended bioassay procedures for bluegill (Lepomis macrochirus, Rafinesque) partial chronic tests. National Water Quality Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota. 12 p.

Bioassay Committee 1971c. Recommended bioassay procedures for brook trout (Salvelinus fontinalis, Mitchill) partial chronic tests. National Water Quality Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota. 12 p.

Boyd, C. E. 1964. Insecticides cause mosquito fish to abort. Progr. Fish. Cult. 26:138.

Brooks, G. T. 1972. Pesticides in Britain. In: Environmental Toxicology of Pesticides. Academic Press, New York. 637 p.

Brungs, W. A. 1969. Chronic toxicity of zinc to the fathead minnow Pimephales promelas, Rafinesque. Trans. Am. Fish. Soc. 98:272-279.

Clemens, H. P. 1950. Life cycle and ecology of Gammarus fasciatus, Say. Hydrobio-Contrib. Vol. 12. Franz Theo. Stone Inst., Ohio State University. 63 p.

Cope, O. B. 1966. Contamination of the freshwater ecosystem by pesticides. J. Appl. Ecol. 3:33-44.

Deuel, C. R., D. C. Haskell, D. R. Brockway, and O. R. Kingsbury 1952. New York State Fish Hatchery feeding chart. N.Y. State Conserv. Dept. Fish. Res. Bull. No. 3, pp. 26.

Drummond, R. A. and W. F. Dawson 1970. An inexpensive method for simulating diel patterns of lighting in the laboratory. Trans. Am. Fish. Soc. 99:434-435.

Eaton, J. G. 1970. Chronic malathion toxicity to the bluegill (Lepomis macrochirus, Rafinesque). Water Research 4: 673-684.

Federal Water Pollution Contracting Administration 1968. Water Quality Criteria. Report of the National Tech. Adm. Comm. to Secr. of the Interior. Fed. Water Poll. Contr. Adm., U.S.D.I. 234 p.

Gakstatter, J. H. and C. M. Weiss 1967. The elimination of DDT-C^{14}, dieldrin-C^{14}, and lindane-C^{14} from fish following a single sublethal dose exposure in aquaria. Trans. Am. Fish. Soc. 96:301-307.

Hansen, D. L., P. R. Parrish, L. I. Lowe, A. J. Wilson Jr., and P. D. Wilson 1971. Chronic toxicity uptake and retention of Aroclor 1254 in two estuarine fishes. Bull. Env. Contam. Toxicol. 6:113-119.

Hermanutz, R. O., L. H. Mueller, and K. D. Kempfert 1973. Captan toxicity to fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus), and brook trout

(Salvelinus fontinalis). J. Fish Res. Bd. Canada 30: 1811-1817.

Hesselberg, R. J. and J. L. Johnson 1972. Column extraction of pesticide from fish food and mud. Bull. Env. Contam. Toxicol. 7:115-120.

Karnak, R. E. and W. J. Collins 1974. The susceptibility to selected insecticides and acetylcholinesterase activity in a laboratory colony of midge larvae, Chironomus tentans. Bull. Env. Contam. Toxicol. 12:62-69.

Leitritz, E. 1959. Trout and Salmon Culture. Fish Bulletin No. 107, State of California, Dept. Fish & Game. 169 p.

Macek, K. J. and S. Korn 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. Bd. Canada 27:1496-1498.

Macek, K. J. and W. A. McAllister 1970. Insecticide susceptibility of some common fish family representatives. Trans. Am. Fish. Soc. 99:20-27.

Matsumura, F. 1972. Current pesticide situation in the United States. In: Environmental Toxicology of Pesticides. Academic Press, New York. 637 p.

McComish, T. S. 1968. Sexual differentiation of bluegills by the urogenital opening. Prog. Fish. Cult. 29:28.

McKim, J. M. and D. A. Benoit 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Canada 28:655-662.

Mount, D. I. 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas, Rafinesque). Water Research 2:215-223.

Mount, D. I. and W. A. Brungs 1967. A simplified dosing apparatus for fish toxicological studies. Water Research 1:21-29.

Mount, D. I. and C. E. Stephen 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96: 185-193.

Mount, D. I. and C. E. Stephan 1969. Chronic toxicity of copper to the fathead minnow (Pimephales promelas) in soft water. J. Fish. Res. Bd. Canada 26:2449-2457.

Nash, R. G. and E. A. Woolson 1967. Persistence of chlorinated-hydrocarbon insecticides in soils. Science 157:924-927.

Pickering, Q. H. and M. H. Gast 1972. Acute and chronic toxicity of cadmium to the fathead minnow (Pimephales promelas). J. Fish. Res. Bd. Canada 29:1099-1106.

Pickering, Q. H. and T. O. Thatcher 1970. The chronic toxicity of linear alkylate sulfonate (LAS) to Pimephales promelas, Rafinesque. J. Water Poll. Cont. Fed. 42: 243-254.

Steel, R. G. D. and J. H. Torrie 1960. Principles and Procedures of Statistics. McGraw Hill, New York. 481 p.

U. S. Environmental Protection Agency 1971. Methods for Organic Pesticides in Water and Wastewater. National Envir. Res. Center, Cincinnati, Ohio. 55 p.

U. S. Environmental Protection Agency 1972. Handbook for Analytical Quality Control in Water and Wastewater Laboratories. National Envir. Res. Center, Cincinnati, Ohio. 114 p.

Wilson, A. J. 1965. Chemical assays. In: U.S. Bur. Comm. Fish. Circ. 247, pp. 6-7. Annual Report of Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida. The fiscal year ending June 30, 1965.